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Transcription Alters Chromosomal Locations of Cohesin in *Saccharomyces cerevisiae*▽†

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In eukaryotic cells, cohesion between sister chromatids allows chromosomes to biorient on the metaphase plate and holds them together until they separate into daughter cells during mitosis. Cohesion is mediated by the cohesin protein complex. Although the association of this complex with particular regions of the genome is highly reproducible, it is unclear what distinguishes a chromosomal region for cohesin association. Since one of the primary locations of cohesin is intergenic regions between converging transcription units, we explored the relationship between transcription and cohesin localization. Chromatin immunoprecipitation followed by hybridization to a microarray (ChIP chip) indicated that transcript elongation into cohesin association sites results in the local disassociation of cohesin. Once transcription is halted, cohesin can reassociate with its original sites, independent of DNA replication and the cohesin loading factor Scc2, although cohesin association with chromosomes in G_2/M is not functional for cohesion. A computer program was developed to systematically identify differences between two ChIP chip data sets. Our results are consistent with a model for cohesin association in which (i) a portion of cohesin can be dynamically loaded and unloaded to accommodate transcription and (ii) the cohesin complex has preferences for features of chromatin that are a reflection of the local transcriptional status. Taken together, our results suggest that cohesion may be degraded by transcription.

Dividing cells must ensure that their chromosomes are copied exactly once and that new cells receive exactly a single copy of each chromosome. Failure to do so can result in aneuploidy, an abnormal number of chromosomes that typically leads to developmental abnormalities or cell death. During DNA replication, sister chromatids are cohered and cohesion is maintained until the metaphase-to-anaphase transition (28, 42). Sister chromatid cohesion facilitates the biorientation of chromosomes along the metaphase spindle and resists the tendency of microtubules to prematurely separate chromosomes once bipolar attachments are established (38). Cohesin also contributes to DNA repair (37, 44) and condensation (15).

In eukaryotic cells, cohesion is mediated by several evolutionarily conserved proteins. The complex itself is composed of two SMC (structural maintenance of chromosomes) subunits, Smc1 and Smc3, and two non-SMC subunits, Mcd1/Scc1 and Scc3. Together, these subunits form a large ring-shaped complex that is essential for cohesion between sister chromatids (14, 16). The loading of cohesin onto chromosomes in G_1 is dependent on the Scc2/4 complex (5, 40). Cohesion is established during DNA replication (42). Cohesion is dissolved at the metaphase-to-anaphase transition by separase, which cleaves Mcd1, resulting in the movement of sister chromatids into separate daughter cells (41, 43). Although the molecular structure of cohesin has been well studied, exactly how this ring-shaped complex interacts with DNA remains unclear. Furthermore, since cohesin is fairly abundant and is present on chromosomes throughout most of the cell cycle, it is interesting to speculate how this complex might accommodate other processes that take place on DNA, such as transcription.

While the cohesin complex reproducibly associates with the same regions of the genome in each cell cycle, cohesin does not appear to bind to a particular DNA motif (13, 25). Cohesin association follows a common pattern on all chromosomes, with regions of intense association in the pericentric/centromere regions and shorter, less intense associations distributed in a semiperiodic manner throughout chromosome arms (3, 13, 25). Intriguingly, cohesin association shows a strong bias toward intergenic regions, where transcription from surrounding units is converging (13, 25). Furthermore, transcript elongation through a cohesin-associated region on a plasmid disrupts the association (13). These results suggest that cohesin may be localized by RNA polymerase sliding or pushing cohesin rings during transcription (25, 35), resulting in a conservation of cohesion. An alternative possibility is that cohesin complexes are assembled and disassembled at loci according to their transcriptional status, with active transcription units forming zones of inhibition for cohesin assembly. According to current models, this could result in the destruction of cohesion, since cohesion must be established concurrent with replication, al-

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TABLE 1. *Saccharomyces cerevisiae* strains used for this study

Strain	Genotype[a]	Reference or source
1827-22D	*MAT***a** *bar1 MCD1-18MYC can1-100 leu2-3,112 his3 ura3-1*	13
JG687	*MAT***a** *bar1 MCD1-6HA can1-100 leu2-3,112 his3 ura3-1*	This study
1827-11B	*MAT***a** *bar1 MCD1-18MYC pGal-MCD1-6HA::URA3 can1-100 leu2-3,112 his3*	Paul Megee
K8250	*MAT***a** *scc2-4 SCC3-18MYC::TRP1 SMC1-6HA::HIS3 Δpep4::LEU2*	5
SEN110	*MAT***a** *bar1 MCD1-18MYC can1-100 leu2-3,112 his3 SMC1-3HA::kan*	This study
CBY601	*MAT***a** *pGal-MCD1-6HA::URA3 his3-11::pCUP1-GFP12-lacI12::HIS3 ade2 scc1-173 armIV-lacO::URA3*	This study
JMH303	*MAT***a** *pGal-MCD1-6HA::URA3 his3-11::pCUP1-GFP12-lacI12::HIS3 ade2 scc1-173 telIV-lacO::LEU2*	This study
SEN111	*MATα pGal-MCD1-6HA::URA3 can1-100 leu2-3,112 his3 scc1-173 SMC3-3FLAG::kan*	This study
NBY292	*MAT***a** *his3-11::pCUP1-GFP12-lacI12::HIS3 ade2 telIV-lacO::LEU2*	2
JG686	*MAT***a** *MCD1-6HA his3-11::pCUP1-GFP12-lacI12::HIS3 ade2 telIV-lacO::LEU2*	This study

[a] All strains are derivatives of W303.

though recent evidence suggests that cohesion can be established without replication (45). The connection between cohesin location and transcription is not understood.

We were interested in the relationship between cohesin location and transcription. We manipulated the transcriptional program of the cell and followed changes in cohesin association. We found that transcriptional induction could disrupt cohesin association. However, at most loci we did not see evidence for an accumulation of cohesin at the 3′ ends of induced genes, which could indicate sliding. In addition, we observed that cohesin could associate with transcriptionally repressed regions, a result not easily explained by the sliding model. Scc2, a component of the Scc2/4 cohesin loading complex (5), is not required for transcription-based association/disassociation. Consistent with a previous report (17), we found that Mcd1 that associates with chromosomes during G_2/M does not participate in functional cohesion. Taken together, these results suggest that cohesion may be degraded by transcription. The flexibility of the pattern of cohesin binding suggests that its association depends on features of chromatin which reflect the local transcriptional status.

MATERIALS AND METHODS

Yeast strains and culture conditions. All *Saccharomyces cerevisiae* strains used in this study are W303 derivatives, and their genotypes are described in Table 1. Cultures were grown in yeast extract-peptone (YP) medium containing 2% glucose (YPD), 2% galactose (YPgal), or 2% raffinose (YPraff) or in minimal medium lacking amino acids. Typical cultures were grown at 30°C in YPD to mid-log phase and arrested in G_1 with α-factor (final concentration, 1 μg/ml) or in metaphase by the addition of nocodazole (final concentration, 15 μg/ml), followed by 1 to 2 h of incubation at 30°C, depending on the strain. For the experiment in Fig. 3, cultures were arrested with nocodazole for 1 h and washed twice with warm YP plus nocodazole to remove any residual YPD. YPgal and nocodazole were added, and cells were incubated for an additional 6 h to ensure complete activation of the galactose promoter.

Flow cytometry. Fluorescence-activated cell sorter (FACS) analysis was performed by pelleting 1 ml of cells and fixing them by the addition of 1 ml 70% ethanol. Cells were then stored at 4°C. Prior to analysis, 0.3 ml of cells was rehydrated in 50 mM sodium citrate and incubated with 0.1 mg/ml RNase at 37°C overnight. Cellular DNA was stained by the addition of 2 μM Sytox Green. Samples were briefly sonicated to break up cellular aggregates. FACS was performed on a CyAnn flow cytometer, and data were analyzed using FloJo software. All FACS-analyzed samples were standardized to an asynchronous culture.

Global gene expression analysis. Aliquots (50 ml) of culture were pelleted at room temperature, flash frozen with liquid nitrogen, and stored at −80°C. Yeast total RNA was extracted from cell pellets by the acid-phenol method followed by ethanol precipitation (36). Polyadenylated RNA was prepared from total RNA by purification with an oligo(dT) cellulose column as described previously (7). RNA quality was assessed on a Bioanalyzer 2100 machine (Agilent). Cy5 and Cy3 dyes were indirectly incorporated into cDNA by using aminoallyl-dUTP (Ambion). Aminoallyl-dUTP was incorporated during reverse transcription of 2 μg of polyadenylated RNA, with priming by a dT_{18} and a dN_9 oligomer. Reverse transcription, coupling, and hybridization protocols were followed as listed on the DeRisi lab website (http://derisilab.ucsf.edu/microarray/protocols.html). Comparative hybridization was carried out using a common polyadenylated RNA reference pool for all samples. Samples were dye swapped, and Cy5- and Cy3-labeled probes were hybridized together to poly-L-lysine-coated microarrays printed with PCR-amplified fragments containing all known and predicted *S. cerevisiae* open reading frames (ORFs) and with custom tiling oligomers of 70 nucleotides in length. Microarrays were scanned with a GenePix 4000B laser scanner (Axon Instruments, Foster City, CA), and the images were analyzed and stored in Acuity software (Molecular Devices Corporation). Data presented were normalized so that the mean of the ratios of medians of all features on the microarray was equal to 1. The data were further filtered to remove any flagged features, features that did not have a feature-to-background intensity of >150, or features with a correlation coefficient of <0.5. Duplicate and dye-swapped data were combined based on their median values and displayed as $\log_2$-normalized binding ratios.

ChIP. For each chromatin immunoprecipitation (ChIP) experiment presented in this study, at least two independent experiments were performed, with similar results. We previously presented evidence that a cohesin ChIP performed with or without antibody on an untagged strain resulted in a stochastic signal (13). Cell cycle arrests were verified by FACS analysis (data not shown). Since cohesin is known to bind strongly to the centromere, as a positive control we compared the levels of enrichment of *CEN3* DNA by semiquantitative PCR for each ChIP sample and for a control ChIP performed without antibody, and in every case, there was a significant enrichment for the *CEN3* sequence when antibody was included. When the DNA recovered from a ChIP performed without antibody was amplified and hybridized to a microarray, no distinct pattern was observed (see Fig. 4). ChIP methods have been published previously and were performed as described previously (13, 23, 27). The control PCR product for each ChIP was amplified using a primer pair that amplified an ~280-bp region specific to the *S. cerevisiae* centromere III region. Primer sequences are shown in Table S7 in the supplemental material.

Genome-wide location analysis. Genome-wide localization analysis was performed in duplicate or triplicate (for some samples). Cy5 and Cy3 labeling of DNA and hybridizations were performed as described previously (4, 12). DNAs from the ChIP assays were subjected to random PCR amplification (4), labeled with Cy5, and competitively hybridized with total genomic DNA labeled with Cy3 as described previously (13). Images were scanned as described above, and the results were stored in both AMAD and Acuity. Data presented were normalized and filtered under the same conditions as those indicated above. Data were analyzed using Microsoft Excel, PeakFinder (13), Axon Acuity, and custom-written R software (R code and instructions are provided in the supplemental material).

Quantitative PCR analysis. Primers used for quantitative PCR are listed in Table S7 in the supplemental material. The real-time reactions were performed using an iCycler machine (Bio-Rad) and iQ SYBR green supermix (Bio-Rad). Dynamic well factors were used, and cycling parameters were as follows: 94°C for

30 s, 50°C for 30 s, and 72°C for 60 s, repeated 40 times. A melting curve analysis was performed, starting at 50°C for 20 s and increasing at a rate of 0.5°C/cycle for 80 cycles. Each run contained a relative standard curve utilizing a serial dilution (1e−1, 1e−2, 1e−3, 1e−4, and 1e−5) of the IP total control DNA and *CEN3* primers. Data analysis was performed with iCycler v3.0 software, and the cycle threshold (C_T) was manually set at a threshold of 500.

Microscopy and one-spot–two-spot analysis. Medium was supplemented with 0.002% adenine to reduce autofluorescence and with $CuSO_4$ to a final concentration of 500 μM to induce LacI-green fluorescent protein (LacI-GFP) from the copper promoter. One milliliter of culture was fixed with 4% paraformaldehyde–3.4% sucrose for 15 min, washed twice with 1 ml of phosphate-buffered saline, and then resuspended in 200 μl of phosphate-buffered saline. An aliquot was used to prepare a slide for fluorescence microscopy on an Axiovert microscope. Twelve to 18 *z* sections were collected as 0.225- to 0.3-μm slices to ensure that we could visualize fluorescence throughout the cell. The exposure time was fixed at 200 ms. Aliquots were stained with DAPI (4′,6′-diamidino-2-phenylindole) for the images shown in Fig. 5 and in Fig. S1 in the supplemental material. Image processing was carried out using AxioVision 4.5 software. Counting of GFP spots was performed manually.

RESULTS

The pattern of cohesin association with chromosomes can be altered by transcriptional changes caused by amino acid starvation. To investigate how transcription affects cohesin association in G_2/M-arrested cells, we manipulated the transcriptional program of the cell using amino acid starvation. Mapping of cohesin was carried out by ChIP followed by hybridization to a microarray (ChIP chip), with Mcd1-18Myc as the target, as previously described (13). A culture grown in rich medium (YPD) was arrested in G_2/M, and then a portion was subjected to 30 min of amino acid starvation. A portion of this culture was transferred back to YPD for 30 min to repress the transcriptional program associated with amino acid starvation. Cell cycle arrest was maintained throughout, as verified by FACS (data not shown). mRNA levels were measured by microarray analysis. The signature transcriptional program induced by amino acid starvation was present (10), albeit at a reduced magnitude, even though the cells were arrested.

The pattern of cohesin association showed several significant changes during amino acid starvation. Overall, the association with many ribosomal protein genes was significantly enhanced ($P < 0.001$), presumably because transcription of ribosomal protein genes is shut down during amino acid starvation (10). Two examples are presented in Fig. 1D and E. Cohesin association with many genes involved in amino acid biosynthesis was significantly depleted ($P < 0.001$), presumably because the transcription of these genes was induced (10). Four examples are presented in Fig. 1A to C and F.

Changes in cohesin association with DNA between rich medium and amino acid starvation conditions were systematically identified using a custom-written R program that calculated the Euclidean distance between two curves over a three-point moving window (the code and instructions are provided in the supplemental material, all ChIP chip data for this experiment are provided in Table S1 in the supplemental material, and all gene expression data are provided in Table S2 in the supplemental material). For the features where Mcd1 displayed a change that was 3 standard deviations greater than the average change (118 features; $P < 0.008$), the correlation with transcription was assessed. For 66% of the features, an increase or decrease in cohesin was correlated with a decrease or increase in transcription (at least 1 standard deviation), respectively. For 20% of the features, there was no associated transcriptional change detected, and for 14% of the features, the transcriptional change was in the same direction as the change in Mcd1 localization. Thus, the majority of the data support the ideas that cohesin association is disrupted by transcriptional induction and that cohesin can associate with transcriptionally repressed regions, although there were exceptions to these general trends.

The centromeric and pericentric regions of each chromosome are normally associated with large amounts of cohesin (3, 13). Although cohesin association with these regions may be subject to different parameters from those for arm cohesin, we found that transcriptional induction caused cohesin to be depleted from these domains, as shown by the disruption of Mcd1 at *CIT2*, which was transcriptionally induced by amino acid starvation and is located 6 kb from *CEN3* (Fig. 1B). When transcription at this region was shut off by transfer back to rich medium, we observed that Mcd1 association increased without any significant change in neighboring regions. The pattern of disappearance and reappearance of Mcd1 in the face of transcriptional induction and repression, respectively, is most consistent with a dynamic association of Mcd1 with chromosomes.

We also examined the pattern of Mcd1 association at regions where transcription was repressed by amino acid starvation. The majority of genes in this class are ribosomal protein genes. We found that if a particular ribosomal protein gene or a neighboring region was associated with Mcd1 in rich medium, this association increased during amino acid starvation (Fig. 1D and E). For instance, the intergenic region where transcription converges from *RPL2B* and *VID28* was a peak of cohesin association in rich medium. When transcription of *RPL2B* was repressed, more Mcd1 appeared to associate with its promoter and the ORF located 5′, i.e., *FAF1*. Following a return to rich medium, this association was disrupted and Mcd1 assumed a preinduction profile. *RPL14B* is located immediately adjacent to *CEN8*, and Mcd1 association increased for this ORF and its promoter during amino acid starvation and then decreased upon transfer back to rich medium. Thus, in terms of Mcd1 association, cohesin at arm regions and pericentric domains appears to respond similarly to transcriptional activation or repression.

One possibility is that Mcd1 responds to transcription by disassociation but that the other subunits do not. A more detailed study of the behavior of each subunit of the complex will be important in the future to elucidate the effect of transcription on the entire complex, although the results shown in Fig. 6 support the idea that the association of the Smc1 subunit is similarly altered by transcription.

The pattern of Mcd1 association with *GAL2* can be altered by galactose-induced transcription. We previously showed that driving transcription through a cohesin-associated region via the $GAL_{1\text{-}10}$ promoter carried on a plasmid resulted in a loss of cohesin association (13). We also showed that induction of transcription at the *GAL2* locus changed the pattern of cohesin at that locus. In glucose-containing medium, there was a single peak of cohesin associated with the promoter and the *GAL2* ORF. However, when cells were continuously cultured in galactose-containing medium, the promoter peak was diminished and a new peak was observed in the *SRL2* ORF, which is located 3′ of the *GAL2* ORF (13). We asked what happened to

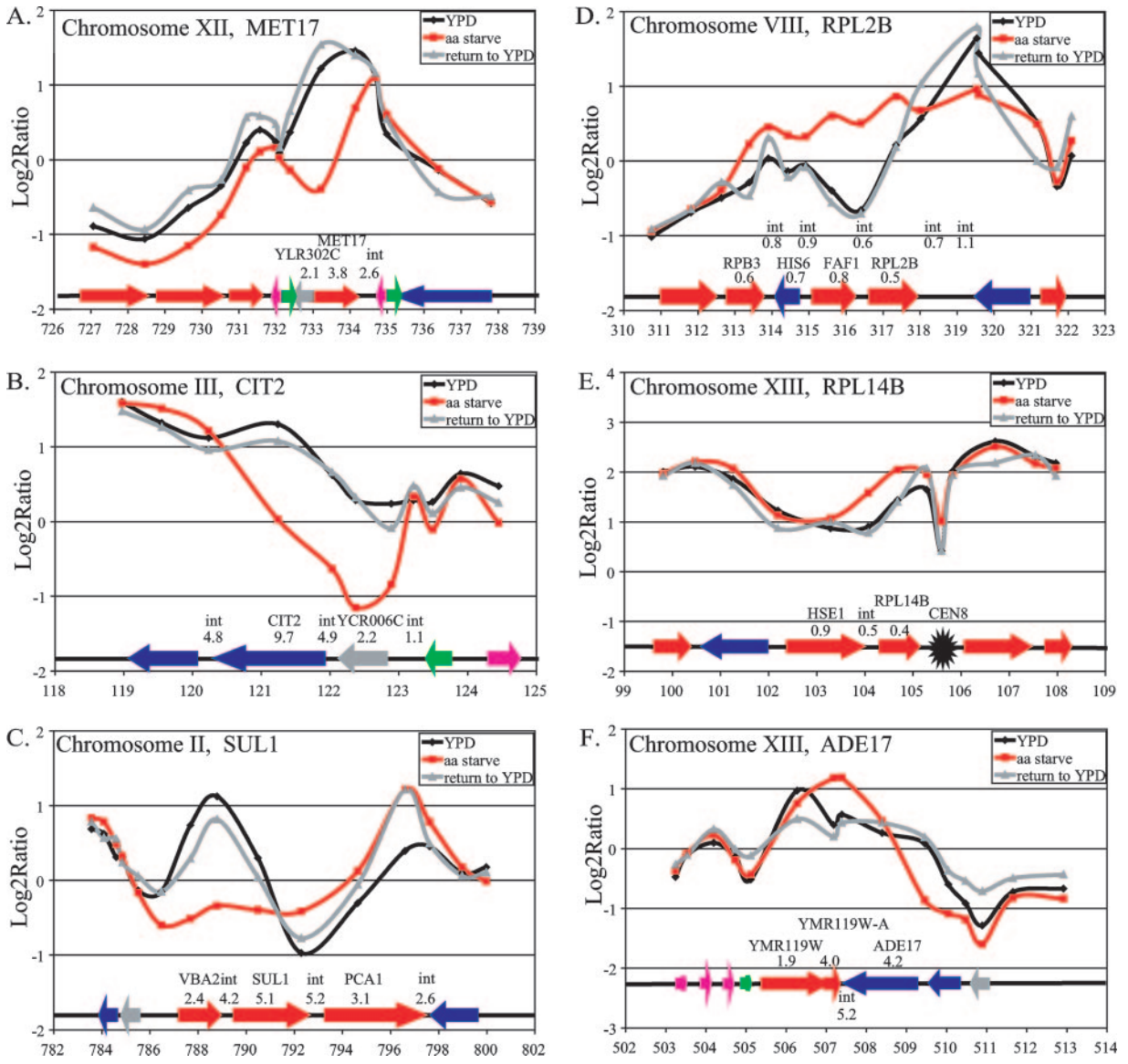

FIG. 1. Alterations of Mcd1-18Myc location during amino acid starvation. Strain 1827-22D was grown in YPD at 30°C and arrested with nocodazole. Following arrest, one-third of the culture was harvested and two-thirds of the culture was transferred to medium lacking amino acids and containing nocodazole. After 30 min, one-third of the culture was harvested and one-third was transferred back to YPD with nocodazole for an additional 30 min. Arrest was maintained throughout the experiment, as verified by FACS analysis. An aliquot of each culture was harvested for gene expression analysis by being frozen in liquid nitrogen (values are indicated above each ORF) and for ChIP chip analysis by formaldehyde cross-linking. The $\log_2$ values of the red/green ratios for the ChIP results (*y* axis) are shown for YPD (black), 30 min of amino acid starvation (red), and return to YPD for 30 min following amino acid starvation (gray). A diagram of each locus is shown on the *x* axis of each graph, and the Saccharomyces Genome Database (SGD) coordinates are given in kb. The *x*-fold change in gene expression from YPD to amino acid starvation is indicated for each feature; numbers of >1 indicate an increase in gene expression during amino acid starvation, and numbers of <1 indicate a decrease in gene expression during amino acid starvation. Genes carried on the Watson strand are shown in red, and genes carried on the Crick strand are shown in blue. Gray arrows are "dubious" ORFs, according to SGD. Pink indicates a long terminal repeat, and green indicates a tRNA. *CEN8* is indicated by a star. (A) *MET17*; (B) *CIT2*; (C) *SUL1*; (D) *RPL2B*; (E) *RPL14B*; (F) *ADE17*.

cohesin in the *GAL2* region in a single cell cycle when transcription was induced (i) prior to DNA replication and (ii) after DNA replication.

A culture growing in YPraff was arrested in G_1 by using alpha factor and then released in the presence of nocodazole and either raffinose or galactose, followed by global mapping of cohesin by ChIP chip analysis. In raffinose, cohesin was found in the promoter region and ORF of *GAL2*, as previously observed (Fig. 2A, red arrow). When galactose was included during S phase, cohesin was instead located 3′ of *GAL2*, in *SRL2* (Fig. 2A, black arrow), similar to the pattern observed previously when cells were continuously cultured in galactose (13). This demonstrates that the transcriptional profile during cohesion establishment can affect the pattern of cohesin association.

Transcription was induced at *GAL2* in nocodazole-arrested

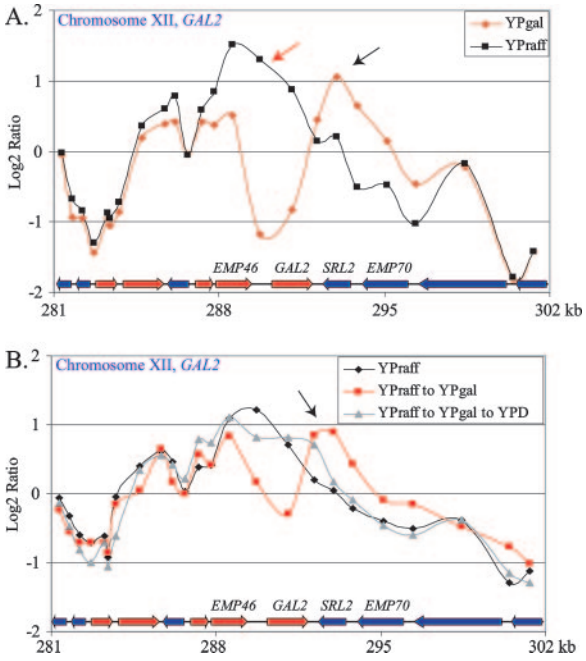

FIG. 2. Galactose-induced transcription at *GAL2* affects cohesin localization. ChIP chip analysis was performed for Mcd1-18Myc in strain 1827-22D under medium conditions that either induced or repressed transcription from the *GAL2* locus. Cell cycle arrests were confirmed in all samples by FACS analysis. The midpoint of each feature is used to represent the $\log_2$ of the median red/green ratio (*y* axis). A depiction of the locus is shown along with coordinates, in kb, for chromosome XII from SGD. (A) A culture grown in YPraff was arrested in G_1 with alpha factor. Following arrest, half of the culture was released into YPgal with nocodazole (red line), and half of the culture was released into YPraff with nocodazole (black line). (B) A culture grown in YPraff was arrested in G_2/M with nocodazole and collected for ChIP (black line). Two-thirds of the culture was transferred to YPgal medium and nocodazole for 1 h and collected for ChIP (red line). Half of this culture was transferred to YPD with nocodazole for 1 h and collected for ChIP (gray line).

cells. In this case, a new peak of cohesin still appeared in *SRL2* (Fig. 2B, black arrow). Surprisingly, when transcription of *GAL2* was repressed by incubating the cells in glucose-containing medium for 1 h, the *SRL2* peak diminished (Fig. 2B, gray line) and the pattern of Mcd1 association more resembled the pretranscriptional induction profile. The only ORF in this region where transcription was significantly affected by the addition of galactose was *GAL2* (data not shown). We concluded that transcription can alter the pattern of cohesin association even in the absence of DNA replication. In several cases of activated transcription that resulted in the disruption of cohesin, we did not observe a new peak 3′ of the induced ORF (Fig. 1). In this sense, *GAL2* may be a somewhat special case with regard to transcription and cohesin localization.

Cohesin localization and galactose-induced transcription. We were interested in understanding the mechanism by which transcription caused a new peak of cohesin association 3′ of *GAL2*, in *SRL2*. One possibility is that the movement of RNA polymerase II through the region slides cohesin rings to the 3′ end of the gene. A second possibility is that the association of the complex is dynamic and that cohesin is removed from the *GAL2* ORF and loaded on *SRL2*. We tested if the cohesin associated with this locus following galactose induction was "old" cohesin or "new" cohesin.

To accomplish this, we used a haploid strain (1827-11B) in which the endogenous copy of Mcd1 was tagged with 18 copies of Myc (Mcd1-18Myc) and an ectopic galactose-inducible copy was tagged with 6 copies of hemagglutinin (HA) (gal-Mcd1-6HA). In order to verify that Mcd-18Myc or Mcd1-6HA did not cause a defect in cohesion, we performed a growth assay comparing strains containing these alleles to a wild-type strain. We found no growth differences (see Fig. S1A in the supplemental material) and no difference in the ability to maintain cohesion (see Fig. S1B in the supplemental material). Strain 1827-11B was initially grown in glucose-containing medium to ensure that no $GAL_{1\text{-}10}$-Mcd1-6HA protein was being made. Cells were arrested using nocodazole, and YPD was replaced with YPgal to induce the transcription of *GAL2* and *$GAL_{1\text{-}10}$-MCD1-6HA*. Western analysis was used to follow the expression of the two epitope-tagged Mcd1 proteins, ChIP was performed to follow their association with DNA, and microarrays were used to follow gene expression. $GAL_{1\text{-}10}$-Mcd1-6HA was strongly expressed by 4 hours according to Western blotting (Fig. 3A). Mcd1-18Myc levels decreased slightly over the course of the arrest in galactose; similar results were obtained when cultures were arrested in glucose, demonstrating that the decrease was not due to the expression of the Mcd1-6HA subunit (data not shown). Expression of mRNA from *GAL2* displayed similar kinetics, that is, maximal expression by 4 h (Fig. 3B). ChIP followed by real-time PCR demonstrated that histone H3 was strongly depleted from the *GAL2* locus by 6 h (see Fig. S2 in the supplemental material). FACS analysis confirmed the G_2/M arrest throughout the experiment (Fig. 3C).

We collected the pattern of Mcd1 association by microarray analysis (for the patterns at 0 and 6 h at *GAL2*, see Fig. 3G; for the patterns at all time points, see Fig. S3 in the supplemental material) and used real-time PCR to quantify the amounts of Mcd1-6HA and Mcd1-18Myc associated with the region upstream of *GAL2* and *SRL2* and with *CEN3* and *FMP32* (cohesin sites where transcription was not affected in galactose compared to that in glucose) in a nocodazole-arrested culture prior to (t = 0 h) and following 6 h of induction with galactose (Fig. 3E and F). For the Mcd1-18Myc present at 6 h, we cannot distinguish whether it was the same Mcd1 that was present at the region at time zero or had been exchanged for Mcd1 present in the soluble nuclear pool. While Mcd1-18Myc was still present at high levels at 6 h (Fig. 3E), a significant amount of Mcd1-6HA was newly associated with these regions (Fig. 3F). These results demonstrate that (i) Mcd1-6HA can associate with chromatin in the absence of DNA replication at *SRL2* as well as in the region upstream of *GAL2*; (ii) Mcd1-6HA can associate with regions where transcription is not affected, such as *CEN3* and *FMP32*; and (iii) Mcd1-6HA localizes to the same regions as Mcd1-18Myc. These results are consistent with de novo association of Mcd1 with chromosomes in G_2/M, as previously shown (17).

While some cohesin may be pushed into *SRL2* from *GAL2* by RNA polymerase II (since we cannot distinguish old versus new Mcd1-18Myc), there is definitely new loading of cohesin (Mcd1-6HA). Taken together, our results suggest that in some cases of transcriptional induction, such as that of *GAL2*, it is

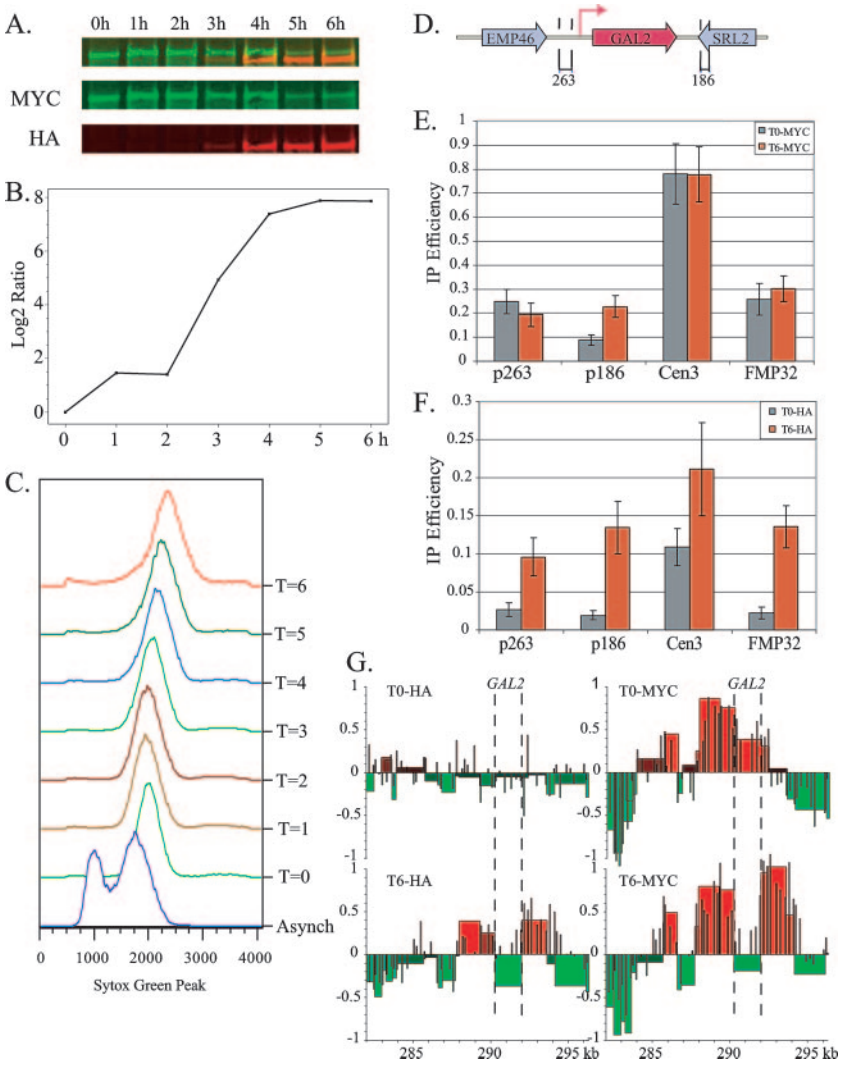

FIG. 3. Galactose-induced Mcd1-6HA associates with *GAL2* when induced in G_2/M. Strain 1827-11B, which contains Mcd1-18Myc under the control of the endogenous Mcd1 promoter and an ectopic copy of Mcd1-6HA under the control of the GAL_{1-10} promoter, was grown in YPD to mid-log phase and arrested with nocodazole. Following arrest, cells were transferred to YPgal medium with nocodazole ($t = 0$ h). Samples were taken every hour for Western analysis, poly(A) mRNA isolation, and FACS analysis. At 6 h, the culture was harvested for ChIP chip analysis. (A) Western blot for GAL_{1-10}-Mcd1-6HA and Mcd1-18Myc. Thirty micrograms of protein was loaded in each lane. (B) mRNA expression of *GAL2*, measured by microarray analysis. (C) FACS analysis. (D) Diagram of PCR products at *GAL2* locus monitored by real-time PCR. (E) Real-time PCR performed in triplicate at time zero and at 6 h for Mcd1-18Myc-associated DNA at *GAL2* as well as *CEN3* and *FMP32*, a cohesin arm site where surrounding transcription is not affected by the switch to galactose medium. (F) Real-time PCR of GAL_{1-10}-Mcd1-6HA-associated DNA. (G) ChIP chip results for Mcd1-18Myc and Mcd1-6HA. This region was monitored on our microarrays by both PCR products and 70-mer oligonucleotides. The width of each product on the microarray is represented by the width of the bar, with the narrowest bars representing the oligonucleotides. The height represents the $\log_2$ value of the median red/green ratio (y axis). The *GAL2* ORF is indicated by vertical dashed lines.

possible that cohesion is maintained locally but that at other loci, such as *MET17* and *CIT2*, where cohesin completely disappears upon transcriptional induction, cohesion may be degraded locally.

When we looked at the pattern of Mcd1-6HA association throughout the genome, we saw that it was present at most sites bound by Mcd1-18Myc (Fig. 4; see Fig. S4 in the supplemental material). Since the number of genes at which cohesin is located and where transcription varies fivefold or more in glucose versus galactose is small (13), transcriptional changes cannot account for the global loading of Mcd1-6HA that we observed. We concluded that the pattern of cohesin association can be altered by transcription but that the new association of Mcd1-6HA with chromosomes does not require transcription. Because Mcd1-6HA associates with the same sites as Mcd1-18Myc, this association appears to be specific to particular preferred regions (not a nonspecific process).

Mcd1 that associates with chromosomes in G_2/M is not functional for cohesion. We asked whether the new Mcd1 that associates with chromosomes in metaphase participates in cohesion. A previous experiment conducted by Haering and colleagues demonstrated that a noncleavable version of Mcd1

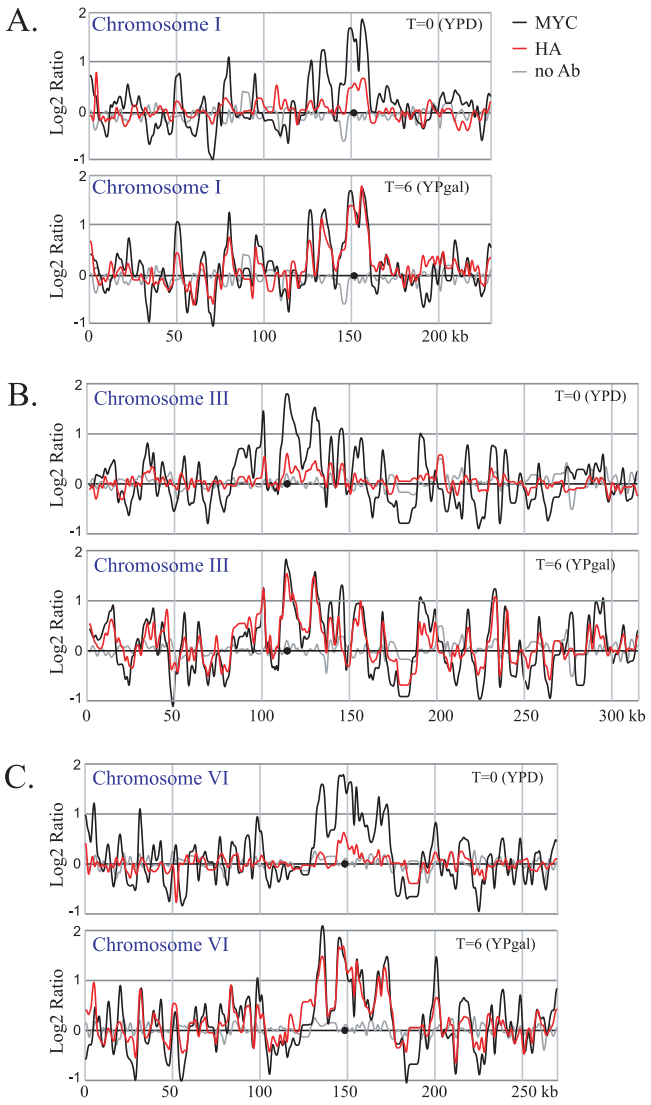

FIG. 4. Galactose-induced Mcd1-6HA associates with the same genomic locations as Mcd1-18Myc when induced in G_2/M. The ChIP patterns of $GAL_{1\text{-}10}$-Mcd1-6HA (red) and Mcd1-18Myc (black) at time zero and following 6 h of induction in galactose were plotted using Peakfinder (13). Data from a ChIP performed without antibody are shown in gray. Vertical gray lines indicate 50-kb intervals. (A) Chromosome I at 0 and 6 h. (B) Chromosome III at 0 and 6 h. (C) Chromosome VI at 0 and 6 h.

expressed in G_2/M could associate with chromosomes but did not prevent chromosome segregation (17). We constructed strains containing a temperature-sensitive allele of Mcd1 (*scc1-173*) along with galactose-inducible Mcd1-6HA. In addition, to visualize chromatid cohesion, we incorporated LacI-GFP and *lacO* repeats at one of two locations, namely, an arm site near *HIM1* on chromosome IV and a telomere on chromosome IV (2). Each strain was grown in YPraff medium and arrested with nocodazole. Galactose was added to half of the culture. The arrest was maintained during the 3-h induction (Fig. 5A). We monitored whether $GAL_{1\text{-}10}$-Mcd1-6HA, expressed during G_2/M (Fig. 5B), could rescue cohesion when cultures were shifted to the nonpermissive temperature of 37°C for 1 h by counting the number of cells with one or two GFP spots (Fig. 5B).

We found that the expression of $GAL_{1\text{-}10}$-Mcd1-6HA did not prevent sister separation at either the arm site or the telomere site (Fig. 5C). The observation that telomere cohesion was more affected than arm cohesion by the shift to 37°C is consistent with a recent report that cohesion is the only force keeping telomeres together (1). Although the numbers of cells with two GFP spots were similar for the raffinose-plus-galactose-induced culture versus the raffinose-only culture prior to the temperature shift, we were surprised to observe that the expression of Mcd1-6HA increased the cohesion defect twofold at both the arm and telomere sites monitored ($P < 0.001$ for the arm and $P = 0.005$ for the telomere). One possible explanation for the increased cohesion defect in the galactose-induced cultures is that the expression of Mcd1-6HA increased the turnover of Mcd1 in cohesin complexes and the turnover rendered the complexes nonfunctional.

In order to assay whether newly expressed Mcd1 could be found to interact with other components of the cohesin complex, we checked whether $GAL_{1\text{-}10}$-Mcd1-6HA was able to coimmunoprecipitate with Smc3-3FLAG when Mcd1-6HA was expressed in nocodazole-arrested cultures grown continuously in YPgal or when galactose was added only during the arrest. We found in both cases that anti-HA antibody was able to coimmunoprecipitate Smc3-3FLAG (Fig. 5D). In addition, in a similar experiment, we subjected the immunoprecipitated material to mass spectrometry, and both Smc1 and Smc3 were detected (data not shown). These results suggest that when Mcd1-6HA is induced during metaphase, it is able to be incorporated into a cohesin complex. Taken together, our results indicate that cohesin complexes that associate with chromatin outside of S phase have the same binding site preferences as those that load during DNA replication but do not efficiently mediate cohesion.

Scc2 is not required for cohesin association or disassociation in G_2/M. Mutations in the human homolog of Scc2/Nipped B have been linked with Cornelia de Lange syndrome and mild precocious sister chromatid separation (20, 22, 39). In budding yeast, Scc2/4 has been shown to be required for the association of the cohesin complex with chromatin following G_1 arrest by cellular fractionation and by ChIP (5, 40). It has been proposed that cohesin inhibits long-range transcriptional activation of the *Drosophila* cut gene and that Scc2/Nipped B facilitates activation by regulating cohesin-chromosome binding (9). We asked if the Scc2/4 complex was required for the alterations observed in response to transcriptional changes that occur during amino acid starvation in G_2/M-arrested cells.

To test the requirement for Scc2 in transcription-induced changes in cohesin, the *scc2-4* temperature-sensitive mutant strain K8250 was grown in YPD at 23°C (permissive temperature) and arrested in G_2/M. Half of the culture was shifted to 33°C for 30 min to inactivate *scc2-4*, as previously described (5, 37). Half of each culture was then subjected to amino acid starvation while being maintained in metaphase arrest. We analyzed the pattern of Smc1-6HA association at the locations previously identified to have the most significant changes during amino acid starvation. We found that changes occurred similarly in some genes at both the permissive and nonpermissive temperatures. For example, the association with several

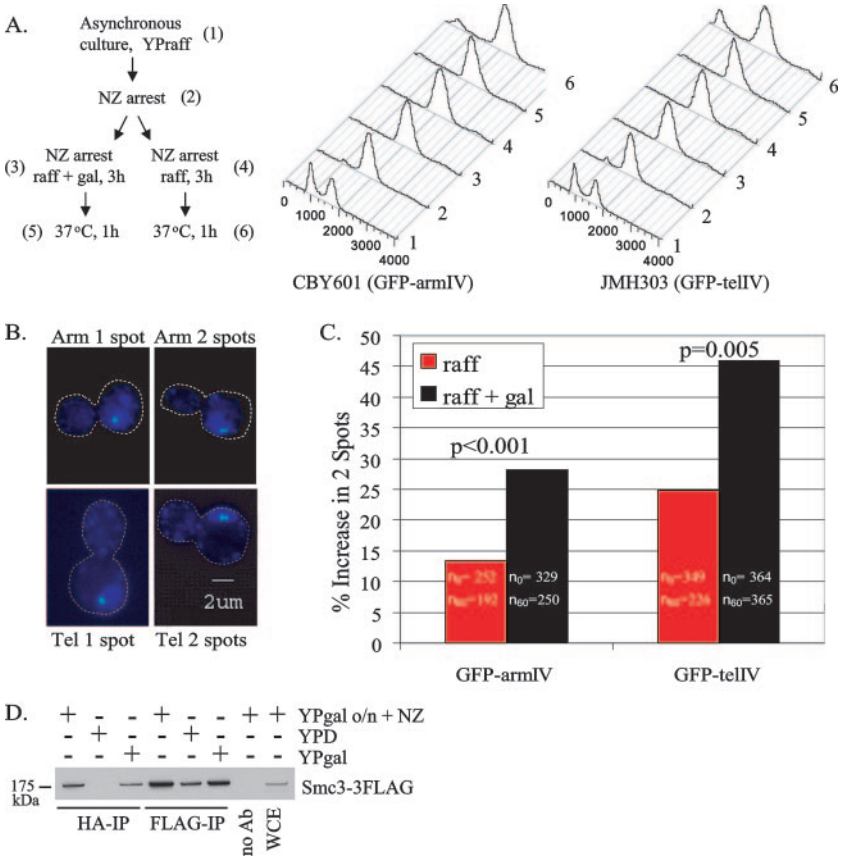

FIG. 5. Galactose-induced Mcd1-6HA that associates with chromosomes in G_2/M is not functional for cohesion. Strains containing either a chromosome arm marked with GFP (CBY601) or a telomere marked with GFP (JMH303) were grown in YPraff at the permissive temperature of 23°C for *mcd1/scc1-173* and arrested with nocodazole (NZ). Following arrest, galactose was added to half of the culture for 3 h, while arrest was maintained. Cultures were shifted to the nonpermissive temperature of 37°C by adding an equal volume of medium at 53°C. (A) Schematic of experiment and FACS profiles confirming the arrest throughout the course of the experiment. The slight peak to the left of the 1N peak in raffinose sample 6 at 60 min is due to cellular debris. (B) Images of strains CBY601 and JMH303 showing either one or two GFP spots and DNA stained with DAPI. (C) Quantitation of the percent increase in two GFP spots following 1 h at 37°C. The numbers of cells for which GFP spots were counted prior to temperature shift (n_0) and following temperature shift (n_{60}) are shown. Prior to the temperature shift, there was no significant difference in the numbers of cells with one GFP spot versus two GFP spots in the YPraff culture compared to the YPraff+gal cultures. (D) Strain SEN111 was grown in YPD and arrested with nocodazole for 2 hours prior to the transfer of half of the culture to YPgal with nocodazole. Cultures were then incubated in YPD or YPgal with nocodazole for 5 hours. As a control, the same strain was grown overnight in YPgal and then arrested with nocodazole. FACS analysis verified all arrests. Immunoprecipitation from whole-cell extract (WCE) was performed with anti-HA antibody, anti-FLAG antibody, or no antibody. The precipitated material was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to a nitrocellulose membrane, and probed with anti-FLAG antibody.

genes occurred normally (*PRS1*/*RPL17A*, *RPL14A*, and *RPL2B*) (Fig. 6B), and disassociation occurred normally at *MET17* (Fig. 6A). First, this result confirms that Smc1 association, in addition to that of Mcd1, can respond to transcription. Second, it demonstrates that Scc2 is not essential for disassociation (Fig. 6A) or association (Fig. 6B) of cohesin in G_2/M-arrested cells.

DISCUSSION

Previous studies have suggested that the localization of the cohesin complex may be connected to the transcriptional status of a location. Here we present evidence that the association of cohesin with chromosomes can dynamically reflect the local transcriptional status of a region. The complex can disassociate from a region in the face of transcriptional induction and can associate with it in the case of transcriptional repression. Cohesin can be deposited at its usual sites independent of DNA replication; however, this newly loaded cohesin is not functional for cohesion. Scc2 function does not appear to be required for the alterations in association of cohesin with DNA due to transcriptional changes. The pattern of cohesin association at some sites is consistent with loading and removal, while the pattern at other sites is consistent with sliding or spreading. We suggest that the cohesin complex itself displays site preferences on chromosomes.

Sliding of cohesin versus loading and unloading. If cohesin can slide locally along chromosomes, then a region where Mcd1 is depleted by transcription will be accompanied by a new peak downstream of transcription, due to sliding. Alternatively, Mcd1 may simply dissociate, in which case no new peak would be observed. In the regions presented in Fig. 1A and B, Mcd1 was depleted over regions that were transcrip-

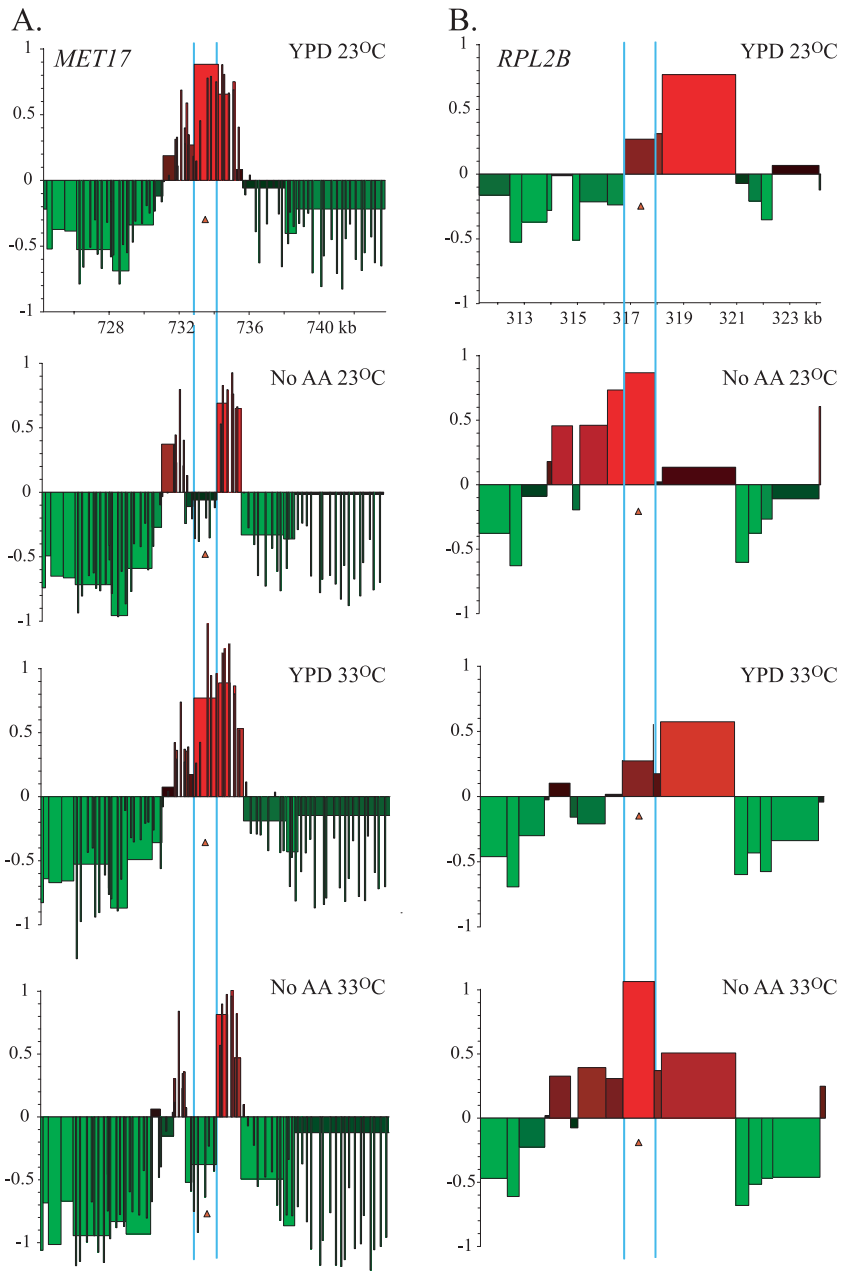

FIG. 6. Scc2 is not required for loading or disruption of Smc1-6HA in G_2/M. Strain K8250, which includes a temperature-sensitive mutation in Scc2 and Smc1-6HA, was grown in YPD at 23°C. Following metaphase arrest for 1.5 h, the culture was split in half and incubated in either YPD at the permissive temperature of 23°C or YPD at the nonpermissive temperature of 33°C for 30 min while maintaining the arrest. Each of these two cultures was then resuspended in YPD with nocodazole or medium lacking amino acids (no AA) and containing nocodazole at their respective temperatures for 1 h. Cells were collected for ChIP. We used microarrays that contained both PCR products and closely spaced oligonucleotide probes for several loci of interest for this experiment. Each vertical bar indicates the $\log_2$ ratio (*y* axis) for a probe on the array, and the width indicates its length. SGD coordinates are shown in kb on the *x* axis. Probes corresponding to the *MET17* ORF (A) and the *RPL2B* ORF (B) are indicated with blue vertical lines and red arrowheads.

tionally induced, but there is no evidence of a new peak located 3′ of transcription. In general, we observed that when transcription was shut off by transfer back to rich medium, Mcd1 resumed its preinduction profile. It seems difficult to reconcile how transcriptional repression could slide cohesin rings to their original location unless one postulates that diffusion of rings can occur in the absence of transcription. These observations suggest that at least for some locations, cohesin is not slid along DNA by RNA polymerase II but, rather, can disassociate from the DNA completely and can reassociate following transcriptional shutoff. Our results for *GAL2* are consistent with either sliding or loading/unloading.

Cohesin and chromatin. Several models for the association of the cohesin complex with DNA have been proposed. Most

models do not require that the cohesin ring is in direct contact with DNA (14, 16–18, 29, 31). However, cohesin reproducibly associates with the same regions of the genome, although there is no obvious sequence specificity to this interaction. It is unclear how many cohesin rings are present at an associated region. Cohesin can be cross-linked to DNA by formaldehyde, which suggests that it is intimately associated with DNA. We propose that the cohesin complex itself prefers to associate with particular chromosomal regions. These preferences do not seem to require the Scc2/4 loading complex. The cohesin complex may be able to distinguish one or more chromatin features, which likely reflects the local transcription status.

The transcriptional status of a region is reflected in several chromatin features, including (i) histone variants, (ii) nucleosome density, (iii) histone modifications, and (iv) supercoiling. Although histone variants (26, 32, 47) and nucleosome density (24, 46) have been mapped genome-wide in yeast, there is nothing obvious that distinguishes intergenic regions between converging transcriptional units with regard to these two chromatin features. The following correlation between a particular histone modification and cohesin association has been demonstrated: when a double-strand break is made, H2A is phosphorylated and cohesin associates with this region to facilitate double-strand break repair (44). Finally, there is some evidence that the promoter regions of genes may transiently accumulate negative supercoils during transcription (21). In this case, terminator regions may accumulate positive supercoils. This could potentially be a preferred substrate for cohesin association. Ivanov and Nasmyth (19) precipitated cohesin associated with a plasmid. The majority was supercoiled, unlike the plasmid precipitated via control proteins (19). In summary, at this time there is not enough evidence to suggest all of the specific features of chromatin that are recognized by cohesin, but it seems likely that local chromatin structure, including histone modification and topology, could play a role in localizing the complex (33).

Cohesin association with DNA may be dynamic. When we induce Mcd1 in G_2/M, we observe that this Mcd1 associates with chromosomes at the usual locations. However, newly associated Mcd1 does not appear to participate in functional cohesion. A previous study found that inducing a noncleavable subunit of Mcd1 during metaphase, which did associate with chromosomes, did not have any effect on chromosome segregation, prompting the authors to suggest that Mcd1 does not turn over in the cohesin complex (17). An alternate interpretation that is more consistent with our observations is that Mcd1 can be exchanged but that this renders the complex nonfunctional for cohesion. Although cohesin that associates with chromosomes in G_2/M is apparently nonfunctional for cohesion, it was recently shown that functional cohesion can be established in G_2 in response to a double-strand break (37). Photobleaching studies recently identified a significant pool of cohesin that interacts dynamically with chromatin in interphase (11), although the nature and function of this cohesin are unknown.

Cohesion must persist from the time of replication until sisters separate from each other prior to cell division. While it might seem counterintuitive to suppose that the association of cohesin with chromosomes could be dynamic, cohesin is located throughout chromosome arms, with a potential site approximately every 11 kb in yeast (3, 13). This level of redundancy could permit any individual complex to be disrupted to allow for transcription without compromising the integrity of cohesion for the entire chromosome. Overexpression of Mcd1 may have artificially increased the turnover of this subunit and rendered a higher-than-normal percentage of complexes nonfunctional, resulting in a cohesion defect. Since we have demonstrated that transcription can disrupt Mcd1 association, it may be that transcription increases subunit turnover and has the effect of locally disrupting cohesion.

The length of time over which the cohesin complex acts may be on the order of an hour (yeast) to 40 years (human oocytes). In the latter case, a lack of subunit turnover would require that the complex established during fetal development in an oocyte would provide cohesion 40 years later, which seems unlikely. Over time, if cohesin subunits turn over, due to transcription or otherwise, the complex may no longer mediate cohesion. If cohesion degrades over time, this could potentially explain why chromosome nondisjunction is more likely as a woman ages.

The link between cohesin, transcription, and Scc2. It is becoming increasingly apparent that cohesin has roles in both cohesion and transcription. The ability of cohesin to accommodate changing transcriptional programs may be especially important during development in more complex organisms. Mutations in Scc2/Nipped B, Smc1, and Smc3 in humans result in a constellation of developmental defects known as Cornelia de Lange syndrome (6, 22, 30). This developmental disorder is probably not the result of defects in cohesion; rather, this syndrome is most likely due to transcriptional defects during embryogenesis (8). Dorsett et al. proposed that Nipped B (the fly homolog of Scc2) aids in the removal of cohesin to allow for long-range promoter-enhancer interactions and subsequent transcription (34). Scc2 is also required for loading in G_1 (5). However, we demonstrate that cohesin can respond to transcription in the absence of Scc2 function, suggesting that Scc2 is not required for local association or disassociation in response to transcription in G_2/M-arrested yeast cells. There are many reasons why our *scc2-4* mutant may not recapitulate the Nipped B mutant phenotype, with the most obvious being that the mutations are different. Regardless, since mutations in cohesin subunits and a loading factor may cause a transcriptional defect in humans, it is important to understand the underlying molecular defect in transcription caused by these mutations. Yeast will likely prove a powerful model system with which to study the link between cohesin and transcription in molecular detail.

Conclusions. In summary, we present evidence that cohesin localization is determined in part by the transcriptional status of a locus. Cohesin association with chromosomes may be dynamic in order to accommodate transcriptional changes. We speculate that this may be accompanied by subunit turnover that leads to local cohesion defects. We propose that certain genomic regions may contain chromatin features, such as histone modifications, that make them preferred locations for cohesin association. The gamut of features that are recognized by cohesin remains to be defined.

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